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(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Fusion Proteins with Immunoglobulin Portions, the
Preparation and Use Thereof

(72) Lauffer, Leander - Germany (Federal Republic of) ;
Oquendo, Patricia - Germany (Federal Republic of) ;
Zettlmeissl, Gerd - Germany (Federal Republic of) ;
Seed, Brian - U.S.A. ;

(73) Behringwerke Aktiengesellschaft - Germany (Federal
Republic of) ;
General Hospital Corporation (The) - U.S.A. ;

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Abstract of th disclosur

Fusion proteins with immunoglobulin portions, the preparation and use thereof

The invention relates to genetically engineered soluble fusion proteins composed of human proteins not belonging to the immunoglobulin family, or of parts thereof, and of various portions of the constant region of immunoglobulin molecules. The functional properties of the two fusion partners are surprisingly retained in the fusion protein.

Description

**Fusion proteins with immunoglobulin portions, the
preparation and use thereof**

The invention relates to genetically engineered soluble
fusion proteins composed of human proteins not belonging
to the immunoglobulin family, or of parts thereof, and of
various portions of the constant region of immunoglobulin
molecules. The functional properties of the two fusion
partners are, surprisingly, retained in the fusion
protein.

EP-A 0 325 262 and EP-A 0 314 317 disclose corresponding
fusion proteins composed of various domains of the CD4
membrane protein of human T cells and of human IgG1
portions. Some of these fusion proteins bind with the
same affinity to the glycoprotein gp120 of human immuno-
deficiency virus as the cell-bound CD4 molecule. The CD4
molecule belongs to the immunoglobulin family and,
consequently, has a very similar tertiary structure to
that of immunoglobulin molecules. This also applies to
the α chain of the T-cell antigen receptor, for which
such fusions have also been described (Gascoigne et al.,
Proc. Natl. Acad. Sci. USA, vol. 84 (1987), 2937-2940).
Hence, on the basis of the very similar domain structure,
in this case retention of the biological activity of the
two fusion partners in the fusion protein was to be
expected.

The human proteins which are, according to the invention,
pr f rably c upl d to th amino terminus f th c nstant
r gion f immunoglobulin d n t belong t th immuno-
globulin family and ar to be assign d to the following
class s: (i) membrane-bound proteins whos extrac llular
domain is wh lly or partly incorporated in th fusion.
Th s are, in particular, thromboplastin and cytokin

r c ptors and growth factor r c ptors, such as th
c llular rec ptorʊ for int rl ukin-4, int rl ukin-7,
tumor necrosis factor, GM-CSF, G-CSF, erythropoietin;
(ii) non-membrane-bound soluble proteins which are wholly
or partly incorporated in the fusion. These are, in
particularly, proteins of therapeutic interest such as,
for example, erythropoietin and other cytokines and
growth factors.

The fusion proteins can be prepared in known pro- and
eukaryotic expression systems, but preferably in mammal-
ian cells (for example CHO, COS and BHK cells).

The fusion proteins according to the invention are, by
reason of their immunoglobulin portion, easy to purify by
affinity chromatography and have improved pharmacokinetic
properties in vivo.

In many cases, the Fc part in fusion protein is
thoroughly advantageous for use in therapy and diagnosis
and thus results, for example, in improved pharma-
cokinetic properties (EP-A 0232 262). On the other hand,
for some uses it would be desirable to be able to delete
the Fc part after the fusion protein has been expressed,
detected and purified in the advantageous manner
described. This is the case when the Fc portion proves to
be a hindrance to use in therapy and diagnosis, for
example when the fusion protein is to be used as antigen
for immunizations.

There are in existence various proteases whose use for
this purpose appears conceivable. Papain and pepsin are
employed, for example, to generate F(ab) fragments from
immunoglobulins (Immunology, ed. Roitt, I. et al., Gower
Medical Publishing, London (1989)), but th y do not
cl ᴀv in a particularly specific manner. Blood coagula-
tion factor Xa by contrast recognis s in a pr t in th
r lativ ly rar t trapeptid sequ nc Il -Glu-Gly-Arg and
performs a hydrolytic cl avage of th pr tein after the

arginin r sidu . S quenc s which c ntain th
d scribed tetrapeptid wer introduc d first by Nagai and
Thogersen in a hybrid protein by genetic engineering
means (Nagai, K. and Thogersen, H.C., Nature, vol. 309
(1984), 810-812). These authors were able to show that
the proteins expressed in B. coli actually are specifi-
cally cleaved by factor Xa. However, there is as yet no
published example of the possibility of such proteins
also being expressed in eukaryotic and, especially, in
animal cells and, after their purification, being cleaved
by factor Xa. However, expression of the proteins
according to the invention in animal cells is preferable
because only in a cell system of this type is there
expected to be secretion of, for example, normally
membrane-bound receptors as fusion partners with
retention of their natural structure and thus of their
biological activity. Secretion into the cell culture
supernatant facilitates the subsequent straightforward
purification of the fusion protein.

The invention thus relates to genetically engineered
soluble fusion proteins composed of human proteins not
belonging to the immunoglobulin family, or of parts
thereof, and of various portions of the constant regions
of heavy or light chains of immunoglobulins 'of various
subclasses (IgG, IgM, IgA, IgE). Preferred as immuno-
globulin is the constant part of the heavy chain of human
IgG, particularly preferably of human IgG1, where fusion
takes place at the hinge region. In a particular embodi-
ment, the Fc part can be removed in a simple way by a
cleavage sequence which is also incorporated and can be
cleaved with factor Xa.

Furth rmor , th inv ntion relat s to proc ss s for th
pr paration f th s fusion proteins by g n tic ngineer-
ing, and to th us th reof for diagnosis and th rapy.

The invention will now be described in relation to th drawings, in which:

Figure 1 shows two oligonucleotide probe molecules used in cloning of thromboplastin cDNA;

Figure 2 shows the nucleotide sequence of clone 2b-Apr5 with the thromboplastin amino acid sequence deduced therefrom;

Figure 3 shows two oligonucleotide sequences which are partially homologous with the sequence of the coding strand (A), and with the non-coding strand (B) of thromboplastin cDNA;

Figure 4 shows the restriction map of plasmid pTF1Fc;

Figure 5 shows two oligonucleotide sequences which are partially homologous with the sequence of the coding strand (A), and with the non-coding strand (B) of the IL-4 receptor cDNA cloned in the vector pDC302/T22-8;

Figure 6 shows the restriction map of plasmid pIL4RFc;

Figure 7 shows two oligonucleotide sequences A and B which are partially homologous with the sequence of the coding strand (A), and with the non-coding strand (B) of the EPO cDNA cloned in the vector pCES; and

Figure 8 shows the restriction map of plasmid pEPOFc.

Finally, th invention is xplained in further examples.

Example 1: Thromboplastin fusion proteins

Blood coagulation is a proc ss of c ntral importance in the human body. There is appropriately delicate regulation of the coagulation cascade, in which a large number of cellular factors and plasma proteins cooperate. These proteins (and their cofactors) in their entirety are called coagulation factors. The final products of the coagulation cascade are thrombin, which induces the aggregation of blood platelets, and fibrin which stabilizes the platelet thrombus. Thrombin catalyzes the formation of fibrin from fibrinogen and itself is formed by limited proteolysis of prothrombin. Activated factor X (factor Xa) is responsible for this step and, in the presence of factor Va and calcium ions, binds to platelet membranes and cleaves prothrombin.

Two ways exist for factor X to be activated, the extrinsic and the intrinsic pathway. In the intrinsic pathway a series of factors is activated by proteolysis in order for each of them to form active proteases. In the extrinsic pathway, there is increased synthesis of thromboplastin (tissue factor) by damaged cells, and it activates factor X, together with factor VIIa and calcium ions. It was formerly assumed that the activity of thromboplastin is confined to this reaction. However, the thromboplastin/VIIa complex also intervenes to activate the intrinsic pathway at the level of factor IX. Thus, a thromboplastin/VIIa complex is one of the most important physiological activators of blood coagulation.

It is therefore conceivable that thromboplastin, apart from its use as diagnostic aid (see below), can also be employed as constitu nt of therapeutic ag nts f r treating inborn or acquir d blood coagulation d ficiencies. Exampl s of this ar chronic hemophilias caus d by a d fici ncy of factors VIII, IX or XI or is acute disturbanc s of blood coagulation as a cons qu nce of, for xampl , liver or kidney dis as . Us of such a

th rapeutic ag nt aft r surgicial int rv ntion would also
be conc ivabl .

Thromboplastin is an integral membrane protein which does
not belong to the immunoglobulin family. Thromboplastin
cDNA sequences have been published by a total of four
groups (Fisher et al., Thromb. Res., vol. 48 (1987),
89-99; Morrisey et al., Cell, vol. 50 (1987), 129-135;
Scarpati et al., Biochemistry, vol. 26 (1987), 5234-5238;
Spicer et al., Proc. Natl. Acad. Sci. USA, vol. 84
(1987), 5148-5152). Thromboplastin cDNA contains an open
reading frame which codes for a polypeptide of 295 amino-
acid residues, of which the 32 N-terminal amino acids act
as signal peptide. Mature thromboplastin comprises
263 amino-acid residues and has a three-domain structure:
i) amino-terminal extracellular domain (219 amino-acid
residues); ii) transmembrane region (23 amino-acid
residues); iii) cytoplasmic domain (carboxyl terminus;
21 amino-acid residues). In the extracellular domain
there are three potential sites for N-glycosylation
(Asn-X-Thr). Thromboplastin is normally glycosylated but
glycosylation does not appear essential for the activity
of the protein (Paborsky et al., Biochemistry, vol. 29
(1989), 8072-8077).

Thromboplastin is required as additive to plasma samples
in diagnostic tests of coagulation. The coagulation
status of the tested person can be found by the one-stage
prothrombin clotting time determination (for example
Quick's test). The thromboplastin required for diagnostic
tests is currently obtained from human tissue, and the
preparation process is difficult to standardize, the
yield is low and considerable amounts of human starting
material (placentae) must be supplied. On the other hand,
it is to be xpected that pr paration of native,
membrane-bound thromboplastin by g n tic ngineering will
also be difficult owing to complex purification proc s-
s s. Th se difficulti s can be avoided by th fusion
according to th inv ntion to immunoglobulin portions.

Th thromboplastin fusion proteins according to the invention ar s cr t d by mammalian c lls (for xampl CHO, BHK, COS c lls) into the cultur medium, purified by affinity chromatography on prot in A-Sepharose and have surprisingly high activity in the one-stage prothrombin clotting time determination.

Cloning of thromboplastin cDNA

The sequence published by Scarpati et al., Biochemistry, vol. 26 (1987), 5234-5238, was used for cloning the thromboplastin cDNA. Two oligonucleotide probe molecules (see Fig. 1) were derived from this. These two probe molecules were used to screen a cDNA bank from human placenta (Grundmann et al., Proc. Natl. Acad. Sci. USA, vol. 83 (1986), 8024-8028).

cDNA clones of various lengths were obtained. One clone, 2b-Apr5, which is used for the subsequent procedure, codes for the same amino-acid sequence as the cDNA described in Scarpati et al. Fig. 2 depicts the total sequence of the clone 2b-Apr5 with the thromboplastin amino-acid sequence deduced therefrom.

Construction of a hybrid plasmid pTF1Fc coding for thromboplastin fusion protein.

The plasmid pCD4E gamma 1 (EP 0 325 262 A2; deposited at the ATCC under the number No. 67610) is used for expression of a fusion protein composed of human CD4 receptor and human IgG1. The DNA sequence coding for the extracellular domain of CD4 is deleted from this plasmid using the restriction enzymes HindIII and BamHI. Only partial cleavage must be carried out with the enzyme HindIII in this cas , in order to cut at only on of the two HindIII sit s c ntained in pCD4E gamma 1 (position 2198). The r sult is an opened vector in which a ukary-otic transcription r gulation s qu nc (promoter) is followed by the open HindIII sit . Th open BamHI sit is

located at th start of th coding r gions for a penta-
peptide link r, followed by the hing and the CH2 and CH3
domains of human IgG1. The reading frame in th BamHI
recognition s qu nc GGATCC is such that GAT is trans-
lated as aspartic acid. DNA amplification with
thermostable DNA polymerase makes it possible to modify
a given sequence in such a way that any desired sequences
are attached at one or both ends. Two oligonucleotides
able to hybridize with sequences in the 5'-untranslated
region (A: 5' GATCGATTAAGCTTCGGAACCCGCTCGATCTCGCCGCC 3')
or

coding region

(B: 5' GCATATCTGGATCCCCGTAGAATATTTCTCTGAATTCCCC 3') of
thromboplastin cDNA were synthesized. Of these, oligo-
nucleotide A is partially homologous with the sequence of
the coding strand, and oligonucleotide B is partially
homologous with the non-coding strand; cf. Fig. 3.

Thus, amplification results in a DNA fragment (827 bp)
which contains (based on the coding strand) at the 5' end
before the start of the coding sequence a HindIII site,
and at the 3' end after the codon for the first three
amino-acid residues of the transmembrane region a BamHI
site. The reading frame in the BamHI cleavage site is
such that ligation with the BamHI site in pCD4E gamma 1
results in a gene fusion with a reading frame continuous
from the initiation codon of the thromboplastin cDNA to
the stop codon of the heavy chain of IgG1. The desired
fragment was obtained and, after treatment with HindIII
and BamHI, ligated into the vector pCD4E gamma 1, as
described above, which had been cut with HindIII
(partially) and BamHI. The resulting plasmid was called
pTF1Fc (Fig. 4).

Transfection of pTF1Fc into mammalian cells

Th fusion prot in ncod d by th plasmid pTF1Fc is
call d pTF1Fc h r inaft r. pTF1Fc was transiently
expr ss d in COS c lls. For this purpose, COS c lls wer

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transfected with pTF1Fc with the aid of DEAE-d xtran
(EP A 0 325 262). Indirect immunofluor scence investiga-
tions revealed that the proportion of transfected cells
was about 25 %. 24 h after transfection, the cells were
transferred into serum-free medium. This cell supernatant
was harvested after a further three days.

Purification of pTF1Fc fusion protein from cell culture
supernatants

170 ml of supernatant from transiently transfected COS
cells were collected overnight in a batch process in a
column containing 0.8 ml of protein A-Sepharose at 4°C,
washed with 10 volumes of washing buffer (50 mM tris
buffer pH 8.6, 150 mM NaCl) and eluted in 0.5 ml frac-
tions with eluting buffer (93:7 100 mM citric acid:
100 mM sodium citrate). The first 9 fractions were
immediately neutralized with 0.1 ml of 2M tris buffer
pH 8.6 in each case and then combined, and the resulting
protein was transferred by three concentration/dilution
cycles in an Amicon microconcentrator (Centricon 30) into
TNE buffer (50 mM tris buffer pH 7.4, 50 mM NaCl, 1 mM
EDTA). The pTF1Fc obtained in this way is pure by
SDS-PAGE electrophoresis (U.K. Lämmli, Nature 227 (1970)
680-685). In the absence of reducing agents it behaves in
the SDS-PAGE like a dimer (about 165 KDa).

Biological activity of purified TF1Fc in the prothrombin
clotting time determination

TF1Fc fusion protein is active in low concentrations
(> 50 ng/ml) in the one-stage prothrombin clotting time
determination (Vinazzer, H. Gerinnungsphysiologie und
Method n im Blutg rinnungslabor (1979), Fish r V rlag
Stuttgart). Th cl tting times achiev d are comparabl
with th clotting times obtained with thromboplastin
isolat d from human placenta.

Example 2: Interleukin-4 receptor fusion proteins

Interleukin-4 (IL-4) is synthesized by T cells and was originally called B-cell growth factor because it is able to stimulate B-cell proliferation. It exerts a large
5   number of effects on these cells. One in particular is the stimulation of synthesis of molecules of immunoglobulin subclasses IgG1 and IgE in activated B cells (Coffmann et al., Immunol. Rev., vol. 102 (1988) 5). In addition, IL-4 also regulates the proliferation and
10   differentiation of T cells and other hemopoietic cells. It thus contributes to the regulation of allergic and other immunological reactions. IL-4 binds with high affinity to a specific receptor. The cDNA which codes for the human IL-4 receptor has been isolated (Idzerda et
15   al., J. Exp. Med., vol. 171 (1990) 861-873). It is evident from analysis of the amino-acid sequence deduced from the cDNA sequence that the IL-4 receptor is composed of a total of 825 amino acids, with the 25 N-terminal amino acids acting as signal peptide. Mature human IL-4
20   receptor is composed of 800 amino acids and, like thromboplastin, has a three-domain structure: i) amino-terminal extracellular domain (207 amino acids); ii) transmembrane region (24 amino acids) and iii) cytoplasmic domain (569 amino acids). In the extra-
25   cellular domain there are six potential sites for N-glycosylation (Asn-X-Thr/Ser). IL-4 receptor has homologies with human IL-6 receptor, with the β-subunit of human IL-2 receptor, with mouse erythropoietin receptor and with rat prolactin receptor (Idzerda et al.,
30   loc. cit.). Thus, like thromboplastin, it is not a member of the immunoglobulin family but is assigned together with the homologous proteins mentioned to the new family of hematopoietin receptors. Members of this family have four cysteine residues and a conserved sequence
35   (Trp-Ser-X-Trp-Ser) in the extracellular domain located near the transmembrane region in common.

On the basis of the described function of the IL-4/IL-4

rec ptor system, th re is a possible therapeutic use of a recombinant form of th IL-4 r c ptor for suppressing IL-4-mediated immune reactions (for xampl transplant r jection reaction, autoimmune dis as s, all rgic r ac-
tions).

The amount of substance required for therapy makes it necessary   to   prepare   such   molecules   by   genetic engineering. Because of the straightforward purification by affinity chromatography and improved pharmacokinetic properties, according to the invention the synthesis of soluble forms of the IL-4 receptor as immunoglobulin fusion protein is particularly advantageous.

The IL-4 receptor fusion proteins are secreted by mammal-ian cells (for example CHO, BHK, COS cells) into the culture medium, purified by affinity chromatography on protein A-Sepharose and have, surprisingly, identical functional properties to the extracellular domain of the intact membrane-bound IL-4 receptor molecule.

Construction of a hybrid plasmid pIL-4RFc coding for IL-4 receptor fusion protein.

Cutting of the plasmid pCD4E gamma1 with XhoI and BamHI results in an opened vector in which the open XhoI site is located downstream from the promoter sequence. The open BamHI site is located at the start of the coding regions for a pentapeptide linker, followed by the hinge and the CH2 and CH3 domains of human IgG1. The reading frame in the BamHI recognition sequence GGATCC is such that GAT is translated as aspartic acid. DNA amplifica-tion with thermostable DNA polymerase makes it possible to modify a given sequence in such a way that any desired s qu nces can be attached at on or both ends. Two oligonucl otid s abl to hybridiz with sequ nc s in th 5'-untranslated r gion

(A: 5' GATCCAGTACTCGAGAGAGAAGCCGGGCGTGGTGGCTCATGC 3') or coding r gion

(B: 5' CTATGACATGGATCCTGCTCGAAGGGCTCCCTGTAGGAGTTGTG 3')
of th IL-4 rec ptor cDNA which is cloned in the vector
pDC302/T22-8 (Idzerda t al., loc. cit.) were
synthesized. Of these, oligonucleotide A is partially
homologous with the sequence of the coding strand, and
oligonucleotide B is partially homologous with the non-
coding strand; cf. Fig. 5. Amplification using thermo-
stable DNA polymerase results in a DNA fragment (836 bp)
which, based on the coding strand, contains at the 5' end
before the start of the coding sequence an XhoI site, and
at the 3' end before the last codon of the extracellular
domain a BamHI site. The reading frame in the BamHI
cleavage site is such that ligation with the BamHI site
in pCD4E gamma 1 results in a gene fusion with a reading
frame continuous from the initiation codon of the IL-4
receptor cDNA to the stop codon of the heavy chain of
IgG1. The desired fragment was obtained and, after
treatment with XhoI and BamHI, ligated into the vector
pCD4E gamma 1, described above, which had been cut with
XhoI/BamHI. The resulting plasmid was called pIL4RFc
(Fig. 6).

Transfection of pIL4RFc into mammalian cells

The fusion protein encoded by the plasmid pIL4RFc is
called pIL4RFc hereinafter. pIL4RFc was transiently
expressed in COS cells. For this purpose, COS cells were
transfected with pIL4RFc with the aid of DEAE-dextran
(EP A 0 325 262). Indirect immunofluorescence investiga-
tions revealed that the proportion of transfected cells
was about 25 %. 24 h after transfection, the cells were
transferred into serum-free medium. This cell supernatant
was harvested after a further three days.

**Purification of IL4RFc fusion protein from cell culture
supernatants**

500 ml of supernatant from transiently transfected COS

c lls were coll cted ov rnight in a batch proc ss in a
column containing 1.6 ml of protein A-Sepharos at 4°C,
wash d with 10 volumes of washing buff r (50 mM tris
buffer pH 8.6, 150 mM NaCl) and eluted in 0.5 ml frac-
tions with eluting buffer (93:7 100 mM citric acid:
100 mM sodium citrate). The first 9 fractions were
immediately neutralized with 0.1 ml of 2M tris buffer
pH 8.6 in each case and then combined, and the resulting
protein was transferred by three concentration/dilution
cycles in an Amicon microconcentrator (Centricon 30) into
TNE buffer (50 mM tris buffer pH 7.4, 50 mM NaCl, 1 mM
EDTA). The IL4RFc obtained in this way is pure by
SDS-PAGE electrophoresis (U.K. Lämmli, Nature 227 (1970)
680-685). In the absence of reducing agents it behaves in
the SDS-PAGE like a dimer (about 150 KDa).

Biological activity of purified IL4RFc

IL4RFc proteins binds ^{125}I-radiolabeled IL-4 with the same
affinity (K_D=0.5 nM) as membrane-bound intact IL-4 recep-
tor. It inhibits the proliferation of IL-4-dependent cell
line CTLLHuIL-4RI clone D (Idzerda et al., loc. cit.) in
concentrations of 10-1000 ng/ml. In addition, it is
outstandingly suitable for developing IL-4 binding assays
because it can be bound via its Fc part to microtiter
plates previously coated with, for example, rabbit anti-
human IgG, and in this form likewise binds its ligands
with high affinity.

Example 3: Erythropoietin fusion proteins

Mature erythropoietin (EPO) is a glycoprotein which is
composed of 166 amino acids and is essential for the
development of erythrocytes. It stimulates the maturation
and the t rminal diff r ntiation of erythroid precursor
c lls. Th cDNA for human EPO has been clon d
(EP-A-0 267 678) and cod s for th 166 amino acids of
matur EPO and a signal peptide of 22 amino acids which
is ss ntial for s cr tion. The cDNA can be used to

pr pare recombinant functional EPO in gen tically manipulat d mammalian cells and the EPO can be employed clinically for th th rapy of anemic manifestations of various etiologies (for example associated with acute renal failure).

Because of the straightforward purification and the improved pharmacokinetic properties, according to the invention synthesis of EPO as immunoglobulin fusion protein is particularly advantageous.

Construction of a hybrid plasmid pEPOFc coding for erythropoietin fusion protein.

This construction was carried out in analogy to that described in Example 2 (section: "Construction of a hybrid plasmid pIL-4RFc coding for IL-4 receptor fusion protein"). Two oligonucleotides able to hybridize with sequences in the vicinity of the initiation codon
(A: 5'GATCGATCTCGAGATGGGGGTGCACGAATGTCCTGCCTGGCTGTGG 3')
and of the stop codon
(B: 5' CTGGAATCGGATCCCCTGTCCTGCAGGCCTCCCCTGTGTACAGC 3')
of the EPO cDNA cloned in the vector pCES (EP-A 0 267 678) were synthesized. Of these, oligonucleotide A is partially homologous with the sequence of the coding strand, and oligonucleotide B is partially homologous with the non-coding strand; cf. Fig. 7. Amplification with thermostable DNA polymerase results in a DNA fragment (598 bp) which, based on the coding strand, contains at the 5' end in front of the initiation codon an XhoI site and in which at the 3' end the codon for the penultimate C-terminal amino acid residue of the EPO (Asp) is present in a BamHI recognition sequence. The reading frame in the BamHI cleavage site is such that ligation with th BamHI site in pCD4E gamma 1 r sults in a g n fusion with a reading frame continuous from th initiation codon of EPO cDNA to th stop codon of the h avy chain of IgG1. Th d sir d fragm nt was obtained and, aft r tr atm nt with XhoI and BamHI, ligat d into

th v ctor pCD4E gamma 1, described abov , which had be n
cut with XhoI/BamHI. Th resulting plasmid was call d
pEPOFc (Fig. 8).

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE
PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A solubl fusion prot in compos d of human proteins
    not belonging to the immunoglobulin family, or of
    parts thereof, and of various portions of immuno-
5   globulin molecules of all subclasses.

2. A fusion protein as claimed in claim 1, wherein the
    immunoglobulin portion is the constant part of the
    heavy chain of human IgG.

3. A fusion protein as claimed in claim 2, wherein the
10 immunoglobulin portion is the constant part of the
    heavy chain of human IgG1 or a protein A-binding
    fragment thereof.

4. A fusion protein as claimed in claim 2 or claim 3,
    wherein the fusion takes place at the hinge region.

15 5. A fusion protein as claimed in claims 1 - 4, wherein
    the protein fused to immunoglobulin is the extra-
    cellular portion of a membrane protein or parts
    thereof.

6. A fusion protein as claimed in claims 1 - 4, wherein
20 the protein fused to immunoglobulin is the extra-
    cellular portion of thromboplastin or parts thereof.

7. A fusion protein as claimed in claims 1 - 4, wherein
    the protein fused to immunoglobulin is the extra-
    cellular portion of a cytokine receptor or growth
25 factor receptor or parts thereof.

8. A fusion protein as claimed in claim 7, wherein the
    prot in fused to immunogl bulin is th extracellular
    portion of IL-4 r c ptor or parts th r of.

9. A fur on prot in as claimed in claim 7, wh r in th
30 prot in fused to immunoglobulin is th xtrac llular
    portion f IL-7 r c ptor or parts ther of.

10. A fusion prot in as claim d in claim 7, wher in th prot in fused to immunoglobulin is the xtrac llular portion of tumor necrosis factor r c ptor or parts thereof.

11. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of G-CSF receptor or parts thereof.

12. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of GM-CSF receptor or parts thereof.

13. A fusion protein as claimed in claim 7, wherein the protein fused to immunoglobulin is the extracellular portion of erythropoietin receptor or parts thereof.

14. A fusion protein as claimed in claims 1 - 4, wherein the protein fused to immunoglobulin is a non-membrane-bound soluble protein or part thereof.

15. A fusion protein as claimed in claim 14, wherein the protein fused to immunoglobulin is a cytokine or growth factor or part thereof.

16. A fusion protein as claimed in claim 15, wherein the protein fused to immunoglobulin is erythropoietin or part thereof.

17. A fusion protein as claimed in claim 15, wherein the protein fused to immunoglobulin is GM-CSF or G-CSF or part thereof.

18. A fusion protein as claimed in claim 15, wher in th prot in fused to immunoglobulin is interl ukin IL-1 to IL-8 or part th reof.

19. A fusion prot in as claim d in any of prec ding claims 1-18, wh r in a factor Xa cleavag sit is additionally ins rted betwe n th immunoglobulin part and the non-immunoglobulin part.

5   20. A process for preparing fusion proteins as claimed in any of claims 1 - 19, which comprises introducing the DNA coding for these constructs into a mammalian cell expression system and, after expression, purifying the produced fusion protein by affinity
10   chromatography via the immunoglobulin portion.

21. The use of the fusion proteins as claimed in any of claims 1 - 19 for diagnosis.

22. The use of the fusion proteins as claimed in any of claims 1 - 19 for therapy.

23.   The fusion protein as claimed in claim 1 and substantially as described h rein.

Fig. 1

```
     GTCGCTCGGACGCTCCTGCTCGGCTGGGTCTTCGCCCAGGTGGCCGGCGCTTCAGGCACT
121  ---------+---------+---------+---------+---------+---------+ 180
     CAGCGAGCCTGCGAGGACGAGCCGACCCAGAAGCGGGTCCACCGGCCGCGAAGTCCGTGA
                                 <*********************************
                                 Oligonucleotide 1


     ACAAATACTGTGGCAGCATATAATTTAACTTGGAAATCAACTAATTTCAAGACAATTTTG
181  ---------+---------+---------+---------+---------+---------+ 240
     TGTTTATGACACCGTCGTATATTAAATTGAACCTTTAGTTGATTAAAGTTCTGTTAAAAC
     ****************************|


===========================================================


                              Oligonucleotide 2
     |*************************************************>
     AACTACTGTTTCAGTGTTCAAGCAGTGATTCCCTCCCGAACAGTTAACCGGAAGAGTACA
721  ---------+---------+---------+---------+---------+---------+ 780
     TTGATGACAAAGTCACAAGTTCGTCACTAAGGGAGGGCTTGTCAATTGGCCTTCTCATGT
```

By: Rogers Bereskin & Parr

~~Fig.~~ 2

```
           10                30                50
GCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCTCTCGGCGAACCCC

           70                90               110
CTCGCACTCCCTCTGGCCGGCCCAGGGCGCCTTCAGCCCAACCTCCCCAGCCCCACGGGC

          130               150               170
GCCACGGAACCCGCTCGATCTCGCCGCCAACTGGTAGACATGGAGACCCCTGCCTGGCCC
                                             MetGluThrProAlaTrpPro

          190               210               230
CGGGTCCCGCGCCCCGAGACCGCCGTCGCTCGGACGCTCCTGCTCGGCTGGGTCTTCGCC
ArgValProArgProGluThrAlaValAlaArgThrLeuLeuLeuGlyTrpValPheAla

          250               270               290
CAGGTGGCCGGCGCTTCAGGCACTACAAATACTGTGGCAGCATATAATTTAACTTGGAAA
GlnValAlaGlyAlaSerGlyThrThrAsnThrValAlaAlaTyrAsnLeuThrTrpLys

          310               330               350
TCAACTAATTTCAAGACAATTTTGGAGTGGGAACCCAAACCCGTCAATCAAGTCTACACT
SerThrAsnPheLysThrIleLeuGluTrpGluProLysProValAsnGlnValTyrThr

          370               390               410
GTTCAAATAAGCACTAAGTCAGGAGATTGGAAAAGCAAATGCTTTTACACAACAGACACA
ValGlnIleSerThrLysSerGlyAspTrpLysSerLysCysPheTyrThrThrAspThr

          430               450               470
GAGTGTGACCTCACCGACGAGATTGTGAAGGATGTGAAGCAGACGTACTTGGCACGGGTC
GluCysAspLeuThrAspGluIleValLysAspValLysGlnThrTyrLeuAlaArgVal

          490               510               530
TTCTCCTACCCGGCAGGGAATGTGGAGAGCACCGGTTCTGCTGGGGAGCCTCTGTATGAG
PheSerTyrProAlaGlyAsnValGluSerThrGlySerAlaGlyGluProLeuTyrGlu

          550               570               590
AACTCCCCAGAGTTCACACCTTACCTGGAGACAAACCTCGGACAGCCAACAATTCAGAGT
AsnSerProGluPheThrProTyrLeuGluThrAsnLeuGlyGlnProThrIleGlnSer
```

By: Roger Brealin + Pa::

Fig. 2 (cont.)

```
          610                  630                  650
TTTGAACAGGTGGGAACAAAAGTGAATGTGACCGTAGAAGATGAACGGACTTTAGTCAGA
PheGluGlnValGlyThrLysValAsnValThrValGluAspGluArgThrLeuValArg

          670                  690                  710
AGGAACAACACTTTCCTAAGCCTCCGGGATGTTTTTGGCAAGGACTTAATTTATACACTT
ArgAsnAsnThrPheLeuSerLeuArgAspValPheGlyLysAspLeuIleTyrThrLeu

          730                  750                  770
TATTATTGGAAATCTTCAAGTTCAGGAAAAGAAAACAGCCAAAACAAACACTAATGAGTTT
TyrTyrTrpLysSerSerSerSerGlyLysLysThrAlaLysThrAsnThrAsnGluPhe

          790                  810                  830
TTGATTGATGTGGATAAAGGAGAAAACTACTGTTTCAGTGTTCAAGCAGTGATTCCCTCC
LeuIleAspValAspLysGlyGluAsnTyrCysPheSerValGlnAlaValIleProSer

          850                  870                  890
CGAACAGTTAACCGGAAGAGTACAGACAGCCCGGTAGAGTGTATGGGCCAGGAGAAAGGG
ArgThrValAsnArgLysSerThrAspSerProValGluCysMetGlyGlnGluLysGly

          910                  930                  950
GAATTCAGAGAAATATTCTACATCATTGGAGCTGTGGTATTTGTGGTCATCATCCTTGTC
GluPheArgGluIlePheTyrIleIleGlyAlaValValPheValValIleIleLeuVal

          970                  990                  1010
ATCATCCTGGCTATATCTCTACACAAGTGTAGAAAGGCAGGAGTGGGGCAGAGCTGGAAG
IleIleLeuAlaIleSerLeuHisLysCysArgLysAlaGlyValGlyGlnSerTrpLys

          1030                 1050                 1070
GAGAACTCCCCACTGAATGTTTCATAAAGGAAGCACTGTTGGAGCTACTGCAAATGCTAT
GluAsnSerProLeuAsnValSer

          1090                 1110                 1130
ATTGCACTGTGACCGAGAACTTTTAAGAGGATAGAATACATGGAAACGCAAATGAGTATT

          1150                 1170                 1190
TCGGAGCATGAAGACCCTGGAGTTCAAAAAACTCTTGATATGACCTGTTATTACCATTAG
```

By: Rigas, Bushkin & Poss

Fig. 2 (cont.)

```
        1210              1230              1250
CATTCTGGTTTTGACATCAGCATTAGTCACTTTGAAATGTAACGAATGGTACTACAACCA


        1270              1290              1310
ATTCCAAGTTTTAATTTTTAACACCATGGCACCTTTTGCACATAACATGCTTTAGATTAT


        1330              1350              1370
ATATTCCGCACTTAAGGATTAACCAGGTCGTCCAAGCAAAAACAAATGGGAAAATGTCTT


        1390              1410              1430
AAAAAATCCTGGGTGGACTTTTGAAAAGCTTTTTTTTTTTTTTTTTTTTTGAGACGGAGTC


        1450              1470              1490
TTGCTCTGTTGCCCAGGCTGGAGTGCAGTAGCACGATCTCGGCTCACTTGCACCCTCCGT


        1510              1530              1550
CTCTCGGGTTCAAGCAATTGTCTGCCTCAGCCTCCCGAGTAGCTGGGATTACAGGTGCGC


        1570              1590              1610
ACTACCACGCCAAGCTAATTTTTGTATTTTTTAGTAGAGATGGGGTTTCACCATCTTGGC


        1630              1650              1670
CAGGCTGGTCTTGAATTCCTGACCTCAGTGATCCACCCACCTTGGCCTCCCAAAGATGCT


        1690              1710              1730
AGTATTATGGGCGTGAACCACCATGCCCAGCCGAAAAGCTTTTGAGGGGCTGACTTCAAT


        1750              1770              1790
CCATGTAGGAAAGTAAAATGGAAGGAAATTGGGTGCATTTCTAGGACTTTTCTAACATAT


        1810              1830              1850
GTCTATAATATAGTGTTTAGGTTCTTTTTTTTTTTCAGGAATACATTTGGAAATTCAAAAC


        1870              1890              1910
AATTGGGCAAACTTTGTATTAATGTGTTAAGTGCAGGAGACATTGGTATTCTGGGCAGCT
```

Fig. 2 (cont.)

```
      1930                1950                1970
TCCTAATATGCTTTACAATCTGCACTTTAACTGACTTAAGTGGCATTAAACATTTGAGAG


      1990                2010                2030
CTAACTATATTTTTATAAGACTACTATACAAACTACAGAGTTTATGATTTAAGGTACTTA


      2050                2070                2090
AAGCTTCTATGGTTGACATTGTATATATAATTTTTTAAAAAGGTTTTTCTATATGGGGAT


      2110                2130                2150
TTTCTATTTATGTAGGTAATATTGTTCTATTTGTATATATTGAGATAATTTATTTAATAT


      2170
ACTTTAAATAAAGGTGACTGGGAATTGTT
```

Fig. 3

```
                    HindIII
        5' GATCGATTAAGCTTCGGAACCCGCTCGATCTCGCCGCC 3' Oligonucleotide A
                  ------|||||||||||||||||||||||||
        AGCCCCACGGGCGCCACGGAACCCGCTCGATCTCGCCGCCAACTGGTAGACATGGAG
  110   ---------+---------+---------+---------+---------+------- 167
        TCGGGGTGCCCGCGGTGCCTTGGGCGAGCTAGAGCGGCGGTTGACCATCTGTACCTC


                                                          MetGlu
        ------------------------------------------------|------
                            5'-untranslated             Start
                                                        Reading frame
                                                        (signal peptide)



■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■


        End of extracellular domain | Start of transmembrane region
        -----------------------------|-----------------------------
        GlnGluLysGlyGluPheArgGluIlePheTyrIleIleGlyAlaVal
        CAGGAGAAAGGGGAATTCAGAGAAATATTCTACATCATTGGAGCTGTGGT
  890   ---------+---------+---------+---------+---------+ 940
        GTCCTCTTTCCCCTTAAGTCTCTTTATAAGATGTAGTAACCTCGACACCA
                 |||||||||||||||||||||||||||
             3' CCCCTTAAGTCTCTTTATAAGATGCCCCTAGGTCTATACG 5' Oligonucleotide B
                                               ------
                            BamHI
```

Fig. 4

Fig. 5

```
              XhoI

5' GATCCAGTACTCGAGAGAGAAGCCGGGCGTGGTGGCTCATGC 3' Oligonucleotide A
           ------||||||||||||||||||||||||||||||||
              AGAGAAGCCGGGCGTGGTGGCTCATGCCTATAATCCCAGCACTTTTGGAGGCTGAGGCGG
       61 ---------+---------+---------+---------+---------+---------+ 120
              TCTCTTCGGCCCGCACCACCGAGTACGGATATTAGGGTCGTGAAAACCTCCGACTCCGCC


              ---------------- 5'-untranslated --------------------



              GCAGATCACTTGAGATCAGGAGTTCGAGACCAGCCTGGTGCCTTGGCATCTCCCAATGGG
      121 ---------+---------+---------+---------+---------+---------+ 180
              CGTCTAGTGAACTCTAGTCCTCAAGCTCTGGTCGGACCACGGAACCGTAGAGGGTTACCC


              --------5'-untranslated------------------------|MetGly


                                                        Start
                              Reading frame (signal peptide)
```
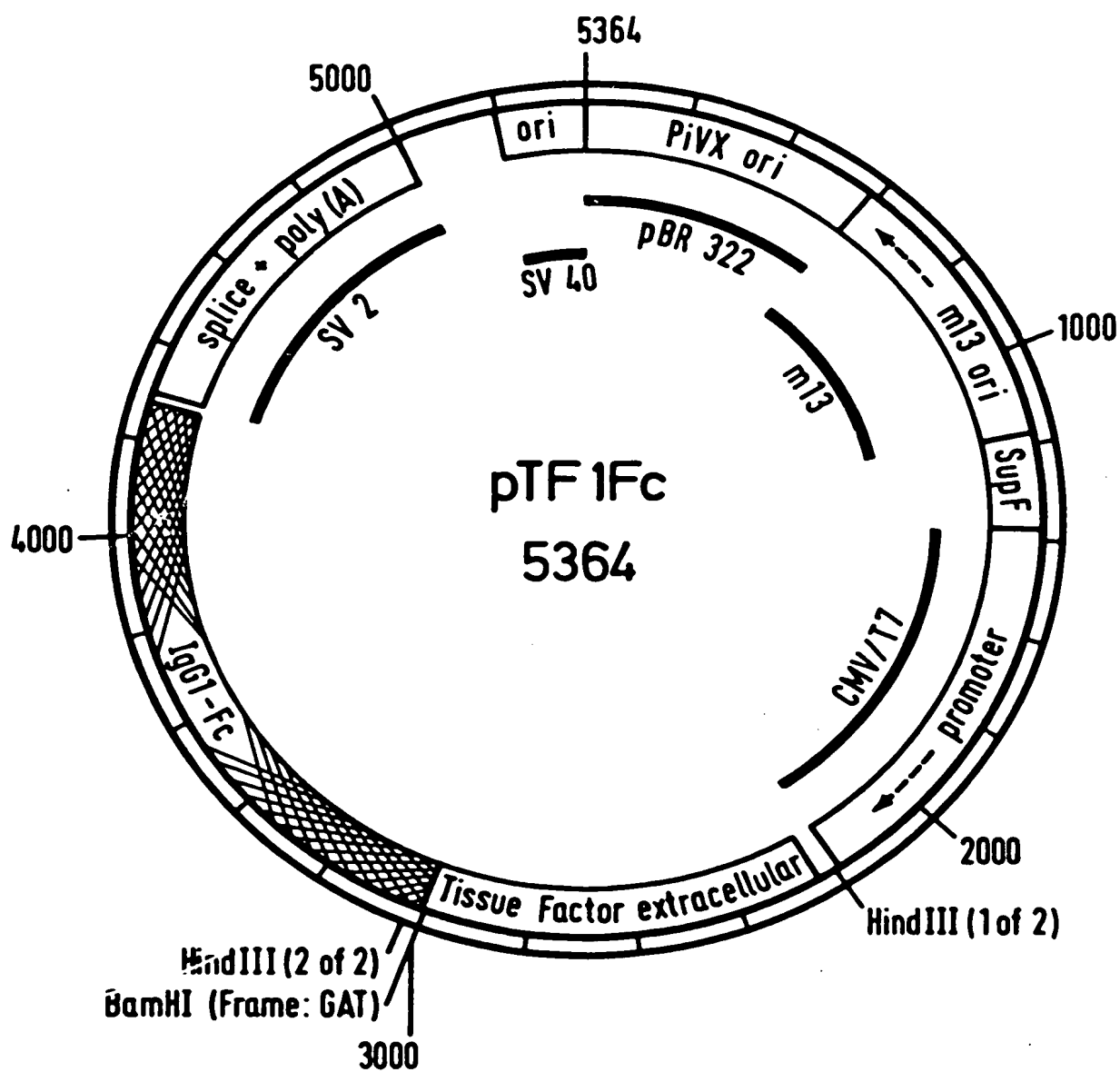
```
■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■
```

```
              End of extracellular domain | Start of transmembrane region
              ----------------------------------|----------------------------
              HisAsnSerTyrArgGluProPheGluGlnHisLeuLeuLeuGlyValSerValSerCys
              CACAACTCCTACAGGGAGCCCTTCGAGCAGCACCTCCTGCTGGGCGTCAGCGTTTCCTGC
      839 -+---------+---------+---------+---------+---------+---------  898
              GTGTTGAGGATGTCCCTCGGGAAGCTCGTCGTGGAGGACGACCCGCAGTCGCAAAGGACG
              |||||||||||||||||||||||||||||||||
       3' GTGTTGAGGATGTCCCTCGGGAAGCTCGTCCTAGGTACAGTATC 5' Oligonucleotide B
                                      ------
                                      BamHI
```

pIL4RFc
5394

Fig. 6

Fig. 1
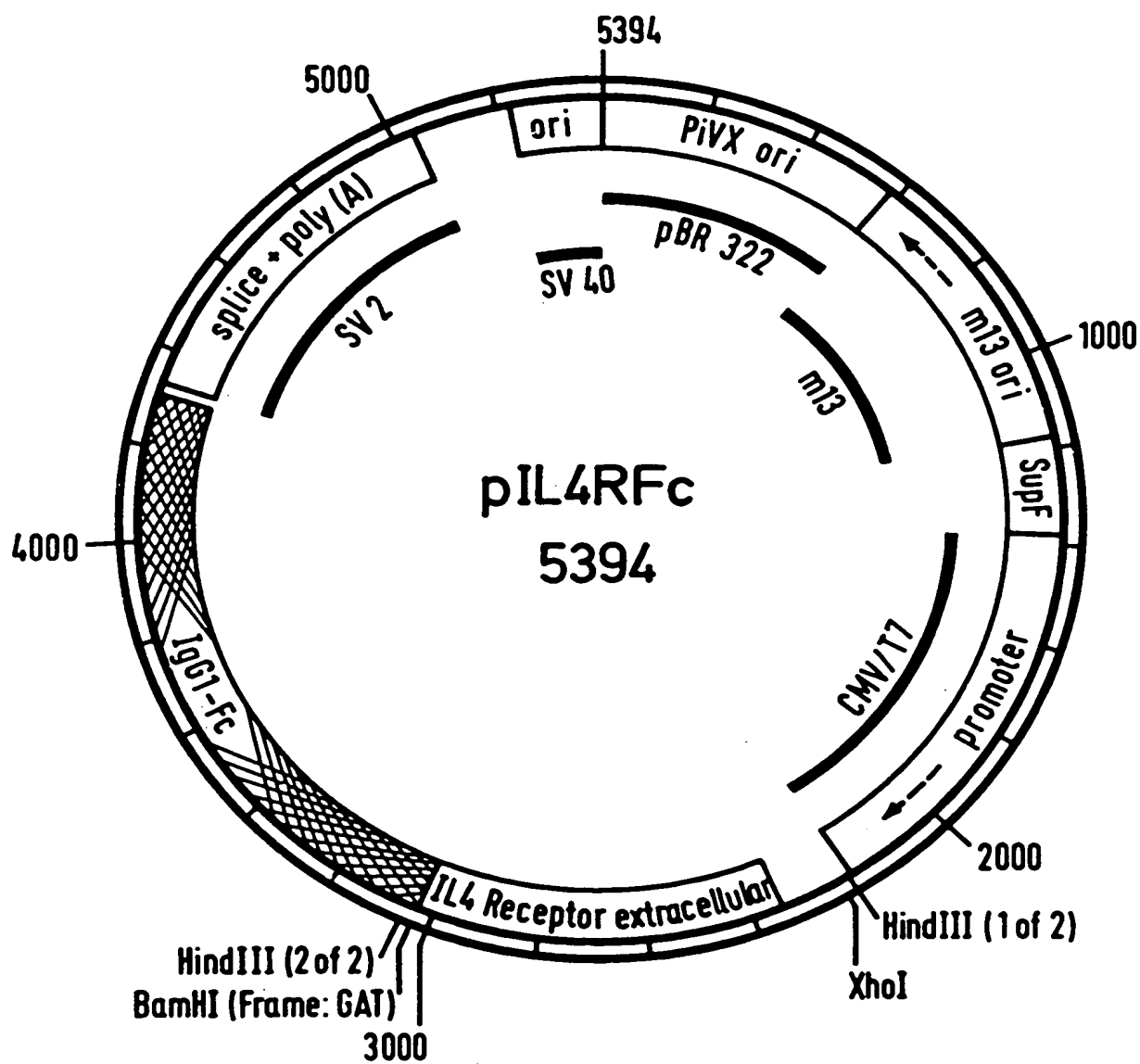
```
        XhoI
5' GATCGATCTCGAGATGGGGGGTGCACGAATGTCCTGCCTGGCTGTGG 3' Oligonucleotide A
        ------|||||||||||||||||||||||||||||||||||
        ATGGGGGGTGCACGAATGTCCTGCCTGGCTGTGGCTTCTCCTGTCCCTGCTGTCG
    182 --------+---------+---------+---------+---------+----- 235
        TACCCCCACGTGCTTACAGGACGGACCGACACCGAAGAGGACAGGGACGACAGC

        MetGlyValHisGluCysProAlaTrpLeuTrpLeuLeuLeuSerLeuLeuSer -

        Start reading frame (signal peptide)
```

■■■

```
        End of reading frame--------------|
        LeuTyrThrGlyGluAlaCysArgThrGlyAspArgEnd
        ------------------------------------|
        GCTGTACACAGGGGAGGCCTGCAGGACAGGGGACAGATGACCAGGTGTGTCCACCTGGGC
    724 ------+---------+---------+---------+---------+---------+--- 783
        CGACATGTGTCCCCTCCGGACGTCCTGTCCCCTGTCTACTGGTCCACACAGGTGGACCCG
        |||||||||||||||||||||||||||||||||||||
     3' CGACATGTGTCCCCTCCGGACGTCCTGTCCCCTAGGCTAAGGTC 5' Oligonucleotide B
        ------
        BamHI
```

Fig. 8